EFFECTS OF SELECTED HERBICIDES AND PLANT HORMONES ON PROTOTHECA WICKERHAMII

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Prototheca is most probably a colourless derivative of a green alga such as Chlorella (Davies, Spencer and Wakelin, 1964; El-Ani, 1967; Migaki, Garner and Imes, 1969). The organism lacks chloroplasts and pyrenoids and has adopted a heterotrophic mode of nutrition (Emmons, Binford and Utz, 1970). It is the cause of the infectious disease protothecosis in mammals (Davies et al., 1964; Klintworth, Fetter and Nielsen, 1968; Frank et al., 1969; van Kruiningen, Garner and Schiefer, 1969; Migaki et al., 1969; Povey et al., 1969; van Kruiningen, 1970). In dogs, abscesses are produced in the viscera with some spread to local lymph nodes (Povey et al., 1969). The disease in cows produces a type of mastitis (Frank et al., 1969; Migaki et al., 1969). In man, protothecosis causes lesions in the skin, but spread to the lymph nodes has been reported (Davies et al., 1964; Davies and Wilkinson, 1967; Klintworth et al., 1968).

To date there is no known cure for protothecosis. Treatment in vitro such as irradiation, cytotoxic agents and antiprotozoal drugs show little apparent effect on the organism (Davies and Wilkinson, 1967). Furthermore, this pathogen does not respond to fungal or bacterial antibiotics administered to human patients (Davies et al., 1964; Klintworth et al., 1968; Mars et al., 1971).

The use of herbicides and plant-growth regulators in the control of undesirable plants has increased in recent years. According to the available nitrogen source, it has been found that P. zopfii may be inhibited by 3-amino-1,2,4-triazole (Casselton, 1964, 1966 and 1967). In the present study the effect of different types of inhibitor on the growth of P. wickerhamii was studied in the hope of recognising a substance that might be of value in the control of protothecosis in mammals.

MATERIALS AND METHODS

The strain of P. wickerhamii used was obtained directly from a dermal lesion at the South African Institute for Medical Research (Mars et al., 1971), and was maintained in a nutrient-broth medium consisting of beef extract 0.1% (w/v), yeast extract 0.2% (w/v), peptone 0.5% (w/v) and 10⁻⁵M thiamine (Anderson, 1944). The cultures were grown at 32°C with a 12-h light-dark cycle of 2000 Lux light (Epel and Krauss, 1966; Emmons et al., 1970). The herbicides selected for study were: maleic hydrazide (MH); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); 2-chloro-4,6-bis(ethylamino)-S-triazine (simazine); sodium chloroauric acid (gold salt), while the plant hormones used were: indolyl-3-acetic acid (IAA); indolyl-3-butyric acid.

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(IBA); indolyl-3-propionic acid (IPA); naphthylacetic acid (NAA); kinetin; gibberellic acid (GA₃) and coconut milk. All the chemicals were dissolved or diluted in deionised water (Vance and Smith, 1969). They were neutralised with sodium hydroxide, autoclaved and added via sterile syringes to the cell cultures.

Cells of *P. wickerhamii* were grown initially in double-strength nutrient concentration. Addition of stock solution of herbicide to the test-tube gave the selected concentration of herbicide (µg per ml) and the normal nutrient concentration (Vance and Smith, 1969). Control tubes were made up with sterile deionised water. Forty-eight-hour cultures of *P. wickerhamii* were inoculated with herbicides and grown for a further 48 h. The cells were fixed in 2.3% glutaraldehyde and then counted in a hemacytometer.

**RESULTS**

Using time-course curves, we discovered that there was a delay in the absorption or activity (or both) of the inhibitory chemicals (fig. 1). This delay (d) was used in a formula compounded to calculate the percentage inhibition of *P. wickerhamii*:

\[
\text{percentage inhibition} = \frac{(C_{96}-C_{48}+d)-(T-C_{48}+d)}{(C_{96}-C_{48}+d)} \times 100
\]

where \(T\) = number of herbicide-treated cells present at 96 h, \(C_{96}\) = number of cells present in the control at 96 h, and \(C_{48}+d\) = number of cells determined from the control after 48 h plus the delay (d).

Fig. 2 shows the results of experiments with herbicides in the concentration range 100–1000 µg per ml. The percentage inhibition with kinetin, which could be used only in the range 10–100 µg per ml because of its insolubility, and with coconut milk (percentage v/v) is shown in fig. 3. Fig. 3 also shows the trend of inhibition obtained with IAA, IBA and IPA at lower concentrations. Concentrations of MH, 2,4,5-T, NAA, GA₃ and simazine of 2000 µg per ml and 4000 µg per ml were also used to treat the cells.
INHIBITION OF PROTOTHECA WICKERHAMII

Time-course studies (fig. 1) indicated a levelling off in the increase in cell number after incubation for 10 h with the inhibitory herbicides. Fig. 2 shows that MH, NAA, 2,4,5-T, simazine, gold salt and GA₃ did not affect growth. IAA, IBA and IPA inhibited cell growth with maximum effect at 400 µg per ml (fig. 2). Coconut milk caused stimulation of growth at the 50% (v/v) level, while kinetin had no effect (fig. 3). IAA, IBA and IPA, studied at the lower concentrations, affected cell growth at 60 µg per ml, the effect being accentuated with further increase in concentration (fig. 2). The general effect of these three chemicals was a steady increase in inhibition of P. wickerhamii from 60 µg per ml to a maximum at 400 µg per ml. None of the other chemicals, at the higher concentrations used, produced any effects.

DISCUSSION

Vance and Smith (1969) were unable to inhibit *Chlorella pyrenoidosa* growth with 2,4,5-T and simazine at a concentration of 1000 µg per ml.

It is known that IAA is present in human urine, and is a normal metabolic product of tryptophan (Anderson, Shimkin and Leake, 1936; Armstrong and Robinson, 1954; Jepson, 1956). Anderson et al. (1936) found the LD50 in
mice given intraperitoneal injections of IAA to be 25 mg per kg, while the LD50 of IBA and IPA was 100 mg per kg. The lower additional requirement of IAA to attain the LD50 concentration may be accounted for by the presence of IAA in mammalian tissues as a product from tryptophan.

Detailed studies of the IAA concentration in human tissues are necessary before it can be ascertained whether cutaneous application of IAA, IBA or IPA will prove effective in the treatment of protothecosis. It is therefore suggested that the use of these compounds to cure this disease merits further investigation.

**SUMMARY**

*Prototheca wickerhamii* was treated *in vitro* with 11 different herbicides and plant hormones. Growth was inhibited by indolyl-3-acetic acid, indolyl-3-butyric acid and indolyl-3-propionic acid at 400 μg per ml. Coconut milk was stimulatory.

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**REFERENCES**


